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Inhalation Toxicity of Vinyl Chloride and Vinylidene Chloride*

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Exposure of mice to 1000 ppm of vinyl chloride (VC), 6 hr/day, 5 days/week, caused some acute deaths with toxic hepatitis and marked tubular necrosis of the renal cortex. Starting the sixth month, mice exposed to 1000, 250, or 50 ppm of VC became lethargic, lost weight quickly, and died. Only a few mice exposed to 50 ppm survived for 12 months. Pulmonary macrophage count was elevated in some mice. There was a high incidence of bronchiolo-alveolar adenoma, mammary gland tumors including ductular adenocarcinoma, squamous and anaplastic cell carcinomas with metastasis to the lung, and hemangiosarcoma in the liver, and, to a lesser extent, in some other organs. The incidence of these tumors quickly increased, and the severity was in direct proportion to the levels of VC and the length of exposure. Malignant lymphoma involving various organs was observed in a few mice.

Rats were more resistant to the toxic effects of VC. Exposure to 1000 ppm slightly depressed the body weight of the females. Exposures of 250 or 1000 ppm caused a number of deaths and hemangiosarcoma in the liver starting the ninth month. Most rats with hepatic hemangiosarcoma also developed hemangiosarcoma in the lung. Hemangiosarcoma occasionally occurred in other tissues of one or two rats exposed to 50 ppm or higher level of VC.

Exposure of mice to 55 ppm of vinylidene chloride (VDC) also caused a few acute deaths and a few hepatic hemangiosarcomas. Inflammatory, degenerative, and mitotic changes occurred in the liver. No mouse exposed to VDC developed any mammary gland tumors. Several mice had bronchioloalveolar adenoma. Exposure of rats to 55 ppm of VDC slightly depressed the body weight. Hemangiosarcoma occurred in the mesenteric lymph node or subcutaneous tissue in two rats.

Introduction

Vinyl chloride (VC) was first prepared more than a century ago, and its fire and explosion hazards are well known. Acute toxicity of VC was first reported in guinea pigs (*1*). As a possible anesthetic agent in dogs, VC was found to cause incoordinated muscular activity of the extremities, cardiac arrhythmias (*2*), and sensitization of the myocardium (*3*). Relatively high concentrations of VC (10–40% in air) for 30 min produced narcosis and/or death in mice, rats, and guinea pigs (*4*). The guinea pigs were found to be more resistant. The main lesions were congestion of the lung with pulmonary edema and hemorrhages in some animals, and congestion of the liver and kidney. Failure of the blood to clot was also observed. Detectable injury of the liver and/or kidney occurred in rats exposed to 100, 250, or 500 ppm but not 50 ppm of VC, 7 hr/day and 5 days/week, for up to 6 months (*5*). Changes in liver and spleen weights, a decrease in leukocytes, and an increase in erythrocytes were observed in rats exposed to 2% VC, 8 hr/day for 3 months (*6*).

Carcinogenic action of VC was first reported in 1971. Male rats (Ar/IRE) exposed to 30,000 ppm of VC, 4 hr/day and 5 days/week for 12 months, developed tumors of the skin, lungs, and bones (*7*).

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Furthermore, Zymbal gland carcinomas, nephroblastoma, hepatic and extrahepatic angiosarcomas were observed in rats and pulmonary tumors, mammary carcinomas, and liver angiosarcomas were observed in mice exposed to as low as 50 ppm of VC, 4 hr/day, 5 days/week, after 7–12 months (*8*). Angiosarcoma of the liver and other hepatic diseases, notably portal fibrosis, were identified among VC polymerization workers (*9–12*). An epidemiological study (*13*) indicated that cancers at multiple sites might be developed in VC and poly(vinyl chloride) workers.

The present study was undertaken to determine the toxic and carcinogenic effects of 50, 250, or 1000 ppm of VC in rats and mice and to define any possible biochemical changes relating to any histological and neoplastic lesions. In addition, one extra chamber was available and used to compare the effects of 55 ppm of vinylidene chloride (VDC).

Material and Methods

Inhalation Chambers and Monitoring

The inhalation chambers are cubical type and made of stainless steel, with a volume of 3.5 m^3. Three chambers were used for 50, 250, or 1000 ppm of VC; one chamber was used for 55 ppm of VDC, and one chamber was used for uncontaminated air as control. VC gas (99.8% pure, Matheson Products) was metered with rotameters into the chamber air supply. VDC (99% pure, Aldrich Company) was heated to 37°C to generate the vapor. The VDC lines and rotameter were heated to 40°C to prevent condensation. Chamber air was initially sampled with a syringe and monitored using a gas chromatograph (Varian-2700) with a flame ionization detector. An automatic sampling system was later used. Periodically, a sample was directed to the gas chromatograph and the readout was processed by a Varian CDS 111 electronic integrator. The integrator was programmed to measure peak area and to calculate chamber concentrations by external standards.

Experimental Design

Albino CD-1 mice and CD rats (Charles River Breeding Lab) about 2 months old were used at the start in these studies. For each species, 360 animals were divided into five groups, each consisting of 36 males and 36 females. Each group of both species was exposed to 50, 250, or 1000 ppm of VC, 55 ppm of VDC, or uncontaminated air for 6 hr/day and 5 days/week. All animals lived in the same stainless steel cages with wire bottoms during exposure and outside of the chambers. Mice were housed four to six per cage and rats two per cage. Pulverized or block laboratory chow (Wayne Manufacturing Company) was provided at all times except during exposure. Water was available *ad libitum*. A 12-hr light cycle was maintained at all times. The temperature in the chamber and in the room averaged 24 ± 1.3°C. The relative humidity ranged 25–60% at the start of the experiment and was later regulated at 50 ± 10%. Four animals of each species, sex, and exposure level were terminated for various laboratory tests, gross and histopathologic examinations at the end of 1, 2, 3, 6, and 9 months; the surviving animals were terminated at the end of 12 months.

Laboratory Evaluations

All animals were observed throughout the study for adverse signs. Feed consumption was recorded weekly and body weight biweekly at a uniform time of day. Heart (mouse) or aortic (rat) blood from four males and four females of each group of each species was collected under anesthesia (ether for mice and sodium pentobarbital for rats) at interim and final terminations. Hematology (RBC, reticulocyte, platelet, WBC and differential counts, nucleated RBC, hematocrit, hemoglobin, methemoglobin, and Heinz bodies) and clinical blood chemistry (SGPT and BUN) were performed on all samples. For rats, prothrombin time, SGOT, alkaline phosphatase, bilirubin, creatinine, LDH, α-HBDH, immunoglobin IgA, IgB-A, IgB-B, and IgM (*14*), total protein (*15*), albumin (*16*), globulin (by difference), and collagen contents in liver and lung (*17, 18*) were also measured. Macrophage counts of pulmonary washings and cytogenic analysis of bone marrow cultures (*19*) were performed on the control, and animals receiving 1000 ppm VC and 55 ppm VDC animals. Limbs from the longest exposed animals were examined for osteoporosis or malacia using a senograph x-ray machine. They were examined for the presence of any bone tumors, any changes in bone density, cortical thickness or striations within the bone cortex, any loss of bone cortex, or any unusually trabecular pattern of the bone. Other specific studies including ^{14}C-thymidine incorporation into DNA (*20*), hepatic aminolevulinic acid (ALA) synthetase (*21*), urinary ALA assays (*22*) and α-fetoprotein (*23*) were performed in mice and /or rats.

When moribund or at termination, all animals were euthanized for necropsy after the collection of blood. Gross examination, especially for any appearance of abnormal growth or other lesions, was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed, sectioned, and stained for microscopic examination. All external and internal tumors were carefully examined and identified histologically.

Results

Mice

General Observations. There were five early deaths, apparently due to the acute effect of the test compounds. Two males and one female exposed to the highest level of VC were found dead between the third and ninth days of exposure; two males exposed to VDC died on the 13th day. They were replaced with healthy mice from the same shipment for the remainder of the experiment. Thereafter, all mice appeared in good health.

During the sixth month, a few mice exposed to VC died or were terminated before their imminent death. The clinical signs included rough hair coat, lethargy, anorexia and rapid weight loss. No death occurred in the control group or the group exposed to VDC. During the seventh through the ninth months, the general health of the mice exposed to VC deteriorated. Additional clinical signs were abdominal distention and/or the appearance of external tumor masses, especially mammary gland tumors in the females. As shown in Table 1, there were numerous deaths or unscheduled terminations, proportional to exposure levels of VC. By the end of the ninth month, all males and females in the group exposed to 1000 ppm and all females exposed to 250 ppm died or were terminated. Additional mice exposed to 250 or 50 ppm died or were terminated during the 10th through 12th months. In the control group, two males died during the eighth and ninth months. One death was due to injury from fighting, the other mouse was found dead with autolysis and the cause of death was unknown. Of the mice exposed to 55 ppm of VDC, two males were terminated during the ninth month and one female during the 10th month. They all had tumors in the liver.

Table 1. Number of deaths and unscheduled terminations of mice exposed to VC or VDC.

Treatment	Sex	Deaths or terminations during exposure month						
		6	7	8	9	10	11	12
Control	M	—	—	1	1	—	—	—
	F	—	—	—	—	—	—	—
VC, 50 ppm	M	1	1	1	1	1	—	1
	F	1	2	4	4	—	2	1
VC, 250 ppm	M	—	1	4	2	1	—	1
	F	3	4	10	—	—	—	—
VC, 1000 ppm	M	2	3	5	3	—	—	—
	F	2	9	6	4	—	—	—
VC, 55 ppm	M	—	—	—	2	—	—	—
	F	—	—	—	—	—	—	1

Body Weight. The weight gains of the male and female mice exposed to 1000 ppm of VC or 55 ppm of VDC were comparable to those of the respective controls during the first 8 months (Fig. 1). Although not shown, the body weights of the mice exposed to 50 or 250 ppm of VC were also not significantly different from the controls. During the ninth month, the body weights of both the males and the females exposed to 1000 ppm began to decline, followed by sudden death.

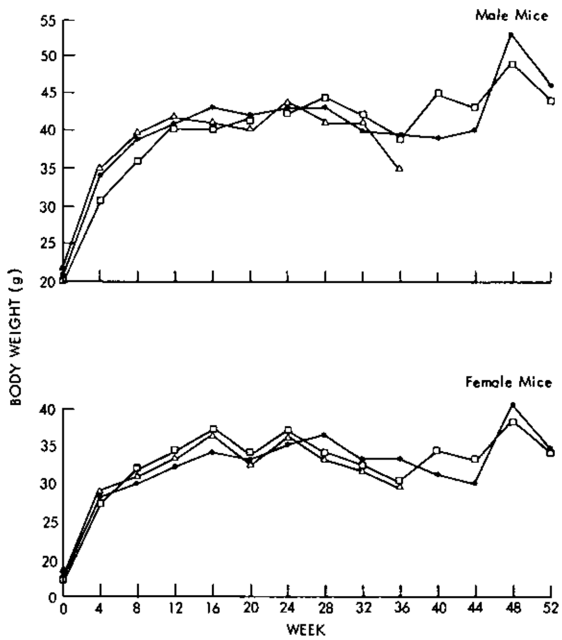

FIGURE 1. Body weights of male and female mice exposed to VC or VDC: (•) control; (Δ) 1000 ppm VC; (□) 55 ppm VDC.

Laboratory Tests. No persistent change was found in the following laboratory results of the male and female mice exposed to VC or VDC as compared with those of the respective controls: hematology, clinical blood chemistry, cytogenic analysis of bone marrow cultures, x-ray examinations of extremities, and serum α-fetoprotein. However, the pulmonary macrophage count in the male mice exposed to 1000 ppm of VC, but not at lower levels, was greater than that of the control mice at the end of the ninth month. The only female mouse in the 250 or 1000 ppm of VC group that was examined at the end of the ninth month also had an elevation of pulmonary macrophage count. At the 12th month, one of two males exposed to 50 ppm also had an elevated macrophage count. There was no survival in the group exposed to 250 or 1000 ppm. The mice with elevated macrophage count also had bronchiolo-alveolar adenoma.

A study of DNA synthesis was undertaken to determine if the observation of an increased number of mitotic figures in the liver of a few mice exposed to VC or VDC could be confirmed at the biochemical level. DNA synthesis, as measured by ^{14}C-thymidine incorporation into DNA, was significantly increased in the livers of male mice exposed to 50 ppm of VC for 11 months (Table 2). However, the livers of these mice did not show any increase in the number of mitotic figures or any evidence of neo- or preneoplastic lesions.

Table 2. ^{14}C-Thymidine Incorporation into Hepatic DNA of male mice exposed to VC for 11 months.

VC level, ppm	^{14}C activity, dpm mg DNA[a]
0	2886 ± 240(8)
50	4232 ± 463(7)[b]

[a]Mean ± SE (number of observations).
[b]Significantly different from the control [two-sample rank test (24)].

Lesions in Early Deaths. Microscopic examination of the five unscheduled deaths (two males and one female exposed to 1000 ppm of VC for 3 to 9 days, and two males exposed to 55 ppm of VDC for 13 days) revealed a number of lesions. These included an acute toxic hepatitis, characterized by focal to marked congestion, and marked diffused coagulation type necrosis of hepatocytes beginning in the centrilobular area. Marked tubular necrosis characterized by pyknosis and eosinophilic granulation of the cytoplasma in the renal cortex was also observed.

Lesions in Late Death. During the eighth to ninth months of exposure, several mitotic figures were observed in the liver of a number of mice exposed to 50 or 1000 ppm of VC. This observation was not apparent in mice terminated at other times. Mice exposed to VDC for 6 to 12 months had several changes in the liver. There were enlarged and basophilic hepatocytes with enlarged nuclei, many of which had large round eosinophilic inclusions; mitotic figures or polyploidy; microfoci of mononuclear cells; focal degeneration and necrosis. The incidence and severity of these lesions progressed with the lengths of exposure. Some of these mice also had hemangiosarcoma.

Tumors. Nearly all the mice that died or were terminated ahead of schedule and many mice that were terminated on schedule at various times developed one or more types of tumors. Bronchiolo-alveolar adenoma first occurred in mice exposed to 1000 or 250 ppm of VC during the second month. The adenoma was characterized by papillary proliferation of alveolar epithelium forming small and well-demarcated, but not capsulated, round nodules. The incidence (Fig. 2) and severity of this tumor increased in direct proportion to the level of VC and to the length of exposure. Only one control male mouse had a bronchiolo-alveolar adenoma during the ninth month. A few small nodules of bronchiolo-alveolar adenoma occurred in six mice exposed to 55 ppm of VDC.

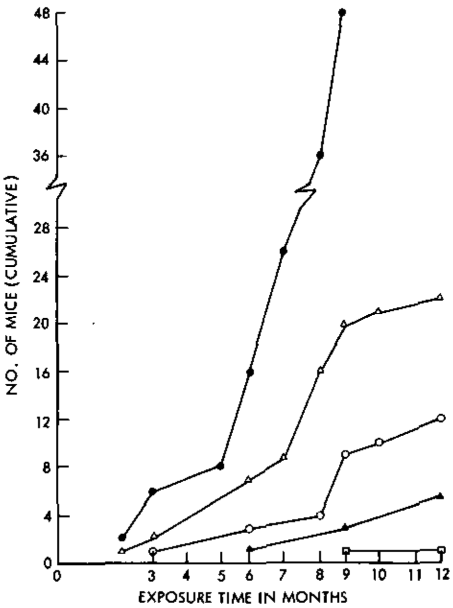

FIGURE 2. Incidence of bronchiolo-alveolar adenoma in male and female mice exposed to VC or VDC: (●) 1000 ppm VC; (△) 250 ppm VC; (○) 50 ppm VC; (▼) 55 ppm VDC; (□) controls.

Hemangiosarcoma occurred in the livers of mice exposed to 1000 or 250 ppm of VC starting the sixth month. The hemangiosarcoma was characterized by moderate to severe proliferation of endothelial cells lining the sinusoids, dilation of the sinusoids, focal hemorrhage forming small to large cavernous blood spaces, invasion of the hepatic parenchyma with neoplastic cells, and mild to severe necrosis. In addition, hemangiosarcoma was occasionally found in other tissues including mammary gland, heart, gastrointestinal tract, pancreas, kidney, epididymis, testis, mesenteric lymph nodes and skeletal muscle. The incidence and severity of the hemangiosarcoma in the liver and the overall incidence in other tissues were related to the level of VC and the length of exposure (Fig. 3). Hepatic hemangiosarcoma occurred in three mice exposed to 55 ppm of VDC. This tumor was not found in any control mice. There were also hemangiomas in the mediastinum of one female exposed to 50 ppm of VC and in the connective tissue adjacent to the salivary gland of one male exposed to 1000 ppm.

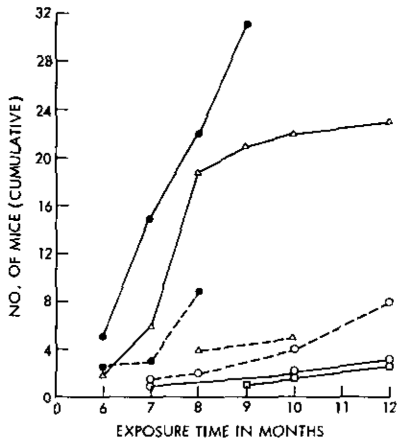

FIGURE 3. Incidence of hemangiosarcoma in male and female mice exposed to VC or VDC: (•) 1000 ppm VC; (Δ) 250 ppm VC; (o) 50 ppm VC; (□) 55 ppm VDC; (——) liver; (--) other organs.

The third major type of tumor was found in the mammary gland of the females. Mammary gland tumors started to occur during the sixth or seventh month. The incidence increased with increases in VC levels and/or length of exposure (Fig. 4). These tumors were composed of ductular adenocarcinoma, squamous cell carcinoma, and/or anaplastic cell carcinoma. The severity of these tumors increased in mice exposed to higher levels of VC and in mice that died or terminated at later dates. In addition, the squamous and/or anaplastic cell carcinomas metastasized to the lungs of a number of mice. This type of tumor was not seen in any control mice or mice exposed to 55 ppm of VDC.

Malignant lymphoma was seen in one female exposed to 50 ppm and one male exposed to 1000 ppm during the sixth month, in two females exposed to 250 ppm during the ninth month, and in one male and three females exposed to 1000 ppm during the ninth month. The malignant lymphomas involved the spleen, liver, lung, kidney, heart, subcutaneous tissue, and/or mammary gland. This tumor was not found in any control mice or mice exposed to 55 ppm of VDC. Three mice exposed to VDC developed hepatoma. Hepatic cell carcinoma, renal adenoma, or skin keratoacanthoma, was observed in one or two mice exposed to 50 or 1000 ppm of VC or 55 ppm of VDC.

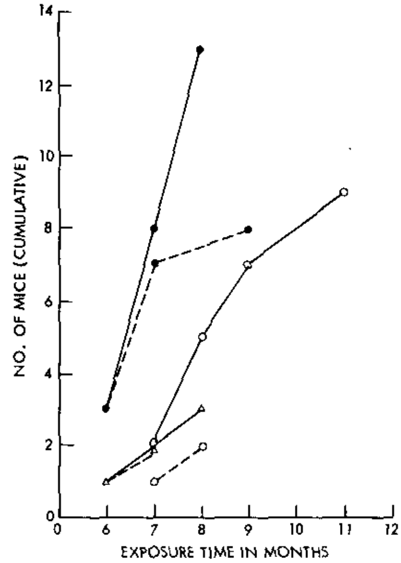

FIGURE 4. Incidence of mammary gland tumors and metastases in the lung in female mice exposed to VC: (•) 1000 ppm; (Δ) 250 ppm; (o) 50 ppm; (——) mammary gland tumor; (--) metastasis to lung.

Rats

General Observations. No remarkable adverse signs were seen in any rats during the first 7 months. Thereafter, a number of rats died or were terminated before their imminent death. In the group exposed to 1000 ppm of VC, eight males and 13 females died or were terminated during the eighth through the 12th months. In the group exposed to 250 ppm, four males and 10 females died or were terminated during the same period. Two females exposed to 50 ppm died. No death occurred in the control group. One female rat exposed to 55 ppm of VDC was terminated. Before death, these rats had rough hair coat, lost muscular tone, were lethargic and lost weight.

Body Weight. The body weights of the female rats exposed to 1000 ppm of VC or 55 ppm of VDC were generally less than that of the female controls after the 4th week and those of the males exposed to 55 ppm of VDC were generally less than that of the male controls after the 24th week (Fig. 5). The body weights of the male and female rats exposed to 50 or 250 ppm of VC, although not shown in Figure 5, were comparable to the controls.

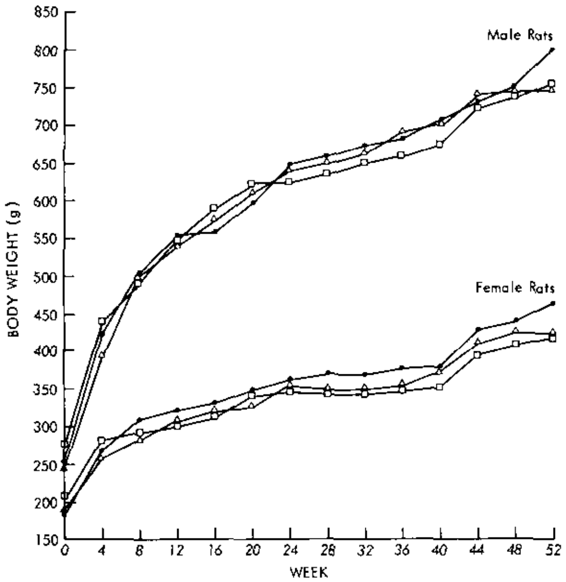

FIGURE 5. Body weights of male and female rats exposed to VC or VDC: (•) control; (Δ) 1000 ppm VC; (□) 55 ppm VDC.

Laboratory Tests. No persistent change was found in the following laboratory results of the male and female rats exposed to VC or VDC as compared with those of the respective controls: hematology, clinical blood chemistry, pulmonary macrophage count, cytogenic analysis of bone marrow culture, x-ray examination of extremities, collagen contents in liver and lung, serum ALA synthetase, urinary ALA level, and serum α-fetoprotein.

Tumors. Hepatic and/or pulmonary hemangiosarcoma occurred in rats exposed to 250 or 1000 ppm of VC during the ninth through the 12th months; the incidence increased with increases in VC levels and length of exposure (Fig. 6). Most of these rats died or were terminated ahead of schedule. The rats with hepatic hemangiosarcoma usually developed pulmonary hemangiosarcoma. In addition, hemangiosarcoma occasionally occurred in other tissues including omentum, mesentery, or subcutaneous tissue of rats exposed to 50, 250, or 1000 ppm of VC. Two rats exposed to 55 ppm of VDC developed hemangiosarcoma in the mesenteric lymph node or subcutaneous tissue. Hemangiosarcoma was not found in the liver, lung or any other organs of any control rats.

A few other tumors occasionally occurred in one or several rats. The tumors included a small nodule of bronchiolo-alveolar adenoma; reticulo-endothelial cell carcinoma or hepatoma in the liver; ductular adenocarcinoma or fibroadenoma in the mammary gland of the female; malignant lymphoma in the spleen or other organs; adenoma in the kidney; squamous cell carcinoma, keratoacanthoma or fibroma in the skin; adenocarcinoma in the sebaceous gland; and chromophobe cell adenoma in the pituitary. These occasional tumors were not related to VC or VDC.

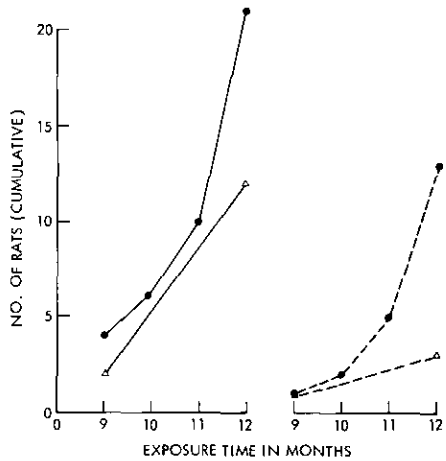

FIGURE 6. Incidence of hemangiosarcoma in male and female rats exposed to VC: (•) 1000 ppm; (Δ) 250 ppm; (——) liver; (--) lung.

Other Microscopic Changes. A mild to markedly severe focal, disseminated vacuolization, probably fatty change, was observed in livers of most of the rats treated with VDC. A few controls and a few rats treated with VC showed this change but to a milder degree.

Discussion and Conclusions

Mice

Exposure to VC or VDC caused some early deaths. Among a total of 36 mice of each sex, two males and one female exposed to 1000 ppm of VC died between the third and ninth days, and two males exposed to 55 ppm of VDC died on the 13th day. Histopathology revealed acute toxic hepatitis and marked tubular necrosis of the renal cortex. Thereafter, all mice appeared in good health. During the sixth month, a few mice exposed to various concentrations of VC had rough hair coat, became lethargic, and lost weight quickly. They died or were terminated before their imminent death. After the seventh month, the general health of the mice exposed to VC deteriorated. A large number of mice had abdominal distention and external tumor masses, especially mammary gland tumors in the females. By the ninth month, both the male and female mice exposed to 1000 ppm of VC and all females exposed to 250 ppm died or were terminated. Only a few mice exposed to 50 ppm survived for 12 months. Two male control mice and two males and one female exposed to 55 ppm of VDC died during the experiment.

Pulmonary macrophage count was elevated in some mice exposed to VC. The macrophage count was elevated earlier in mice exposed to 1000 or 250 ppm than in mice exposed to 50 ppm. The mice with elevated macrophage count also had bronchiolo-alveolar adenoma. However, not all mice with bronchiolo-alveolar adenoma had the elevation of macrophage count. The relationship of macrophage count and this pulmonary tumor needs further investigation.

A moderate number of mitotic figures were observed in the liver of mice exposed to VC during the ninth month. DNA synthesis, as measured by ^{14}C-thymidine incorporation into DNA, was significantly increased in the livers of male mice exposed to 50 ppm of VC for 11 months. However, the mitotic figures were not observed in the livers of these mice, nor was there any evidence of neoplastic and preneoplastic lesions. Further studies are underway to determine if the histopathological observation could be related to changes at the biochemical level.

A number of lesions occurred in the liver of mice exposed to 55 ppm of VDC. The lesions included enlarged and basophilic hepatocytes, enlarged nuclei with eosinophilic inclusions, mitotic figures or polyploidy, microfoci of mononuclear cells, focal degeneration, and necrosis. The significance of these lesions as related to the hemangiosarcoma or other liver tumors may be of a "preneoplastic" nature. Since only a few mice, as compared to the number of those with these preneoplastic lesions, developed the definite neoplasms, further study with mice exposed to high levels of VDC is needed to establish any definite conclusions.

Exposure to 50, 250 or 1000 ppm of VC, 6 hr/day and 5 days/week, caused bronchiolo-alveolar adenomas, mammary gland tumors, and hemangiosarcomas. The squamous and anaplastic cell carcinomas of the mammary gland metastasized to the lung. Hemangiosarcomas first occurred in the liver and then in other organs. The incidence of these tumors quickly increased, especially in mice exposed to 1000 or 250 ppm. The severity was in direct proportion to the levels of VC and the length of exposure. In addition, a few mice exposed to VC also developed malignant lymphomas. Bronchioloalveolar adenomas and hepatic hemangiosarcomas also occurred in some mice exposed to 55 ppm of VDC. The pathogenesis of these tumors was described elsewhere (*25*).

Rats

Rats were more resistant to the toxic and carcinogenic effects of VC or VDC. Exposure to 50, 250, or 1000 ppm of VC or 55 ppm of VDC did not cause any acute deaths, or any persistant changes in hematology or clinical laboratory results. However, exposure to 1000 ppm of VC or 55 ppm of VDC slightly depressed the body weight of the females or both sexes. In addition, a number of deaths occurred in the rats exposed to 250 or 1000 ppm of VC or 55 ppm of VDC starting the 9th month.

Exposure to 250 or 1000 ppm of VC caused hemangiosarcoma in the liver starting the ninth month. In contrast to the mice, many of the rats with hepatic hemangiosarcomas also developed hemangiosarcomas in the lung. Two rats exposed to 55 ppm of VDC developed hemangiosarcomas in the mesenteric lymph node or subcutaneous tissue. The livers of most rats exposed to VDC had a mild or markedly severe focal, disseminated vacuolization, probably fatty change. The significance of this change as related to the exposure of VDC is not understood.

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